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Lower limbic metabotropic glutamate receptor 5 availability in alcohol dependence

Running Title: Lower mGlu5 in alcohol dependence

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ABSTRACT

Animal studies suggest an important role for the metabotropic glutamate receptor subtype 5 (mGlu5) in the pathophysiology of alcohol dependence, but direct human evidence is lacking. The goal of this study was to investigate cerebral mGlu5 availability in alcohol-dependent patients versus controls using ^{18}F-FPEB positron emission tomography. **Methods:** Dynamic 90 minute ^{18}F-FPEB scans combined with arterial blood sampling were acquired in 16 recently abstinent alcohol-dependent patients and 32 age-matched controls. Regional mGlu5 availability was quantified by the ^{18}F-FPEB total distribution volume using both a voxel-by-voxel and a volume-of-interest analysis with partial-volume effect correction. Alcohol consumption within the last 3 months was assessed by questionnaires and by hair ethyl glucuronide analysis. Craving was assessed using the Desire for Alcohol Questionnaire. **Results:** mGlu5 availability was lower in mainly limbic regions of alcohol-dependent patients compared to controls (P<0.05, family-wise error corrected), ranging from 14% in the posterior cingulate cortex up to 36% in the caudate nucleus. Lower mGlu5 availability was associated with higher hair ethyl glucuronide levels for most regions, and was related to a lower level of craving specifically in the middle frontal gyrus, cingulate cortex and inferolateral temporal lobe. **Conclusion:** These findings provide first human *in vivo* evidence for a role of limbic mGlu5 in the pathophysiology of alcohol dependence, possibly involved in a compensatory mechanism helping to reduce craving during abstinence.

KEY WORDS : PET; mGlu5, alcohol dependence, craving

INTRODUCTION

In 2009, Peter W. Kalivas proposed '*the glutamate homeostasis hypothesis of addiction*'. According to it, the transition from controlled drug use to dependence involves long-lasting glutamatergic neuroadaptations in corticolimbic areas (*1*). Among others, impaired mGlu5-dependent signaling is hypothesized to represent a key component of these adaptations. The mGlu5 is a widely distributed G11/q-protein coupled receptor of the human brain, with highest expression in corticolimbic areas (*2*). Mainly located on post-synaptic terminals, mGlu5 is integral to synaptic plasticity processes partly via its functional positive coupling with the N-methyl-D-aspartate receptor (*3*). Kalivas's theory posit that chronic drug administration results in a mGlu5 downregulation, representing a compensatory adaptation helping to reduce compulsive drug-seeking. Hence, mGlu5 has been proposed as a promising target for the treatment of several types of substance dependence (*4*).

Alcohol dependence is a major health concern associated with high societal costs worldwide (*5*), and with high relapse rates despite available therapies. Therefore, understanding the neural mechanisms that mediate the transition from controlled use to dependence in humans remains a priority of alcohol research.

To date, few preclinical studies investigated the effect of chronic alcohol administration on mGlu5 density, revealing either a mGlu5 hypo-expression in the hippocampus (*6*), an over-expression in the amygdala (*7*), or no alterations (*8*) or over-expression (*9*) in the ventral striatum. In contrast, extensive research showed that blocking mGlu5 signaling by genetic or pharmacological interventions reduces alcohol rewarding properties (*10-14*) as well as the severity of withdrawal and relapse behaviors following alcohol deprivation in rodents (*13,15-19*).

In the last 10 years, the highly selective and potent mGlu5 positron emission tomography (PET) radioligand ^{18}F-FPEB has allowed quantification of mGlu5 availability (*20*), thereby providing a

unique opportunity to examine how mGlu5 may adapt across the different stages of alcohol dependence. Noteworthy, ^{18}F-FPEB PET has shown that limbic mGlu5 positively relates to novelty-seeking (*21*), a heritable temperament trait associated with increased susceptibility to drug dependence (*22*). Moreover, genetic studies have demonstrated that individuals with specific mGlu5 polymorphisms are at higher risk for developing alcohol dependence (*23*,*24*). However, the *in vivo* status of mGlu5 availability in alcohol-dependent patients and its possible relation to clinical outcome is unknown.

Here, we investigated mGlu5 availability in recently abstinent alcohol-dependent patients compared to controls using ^{18}F-FPEB PET. Considering the largely shared neurobehavioral deficits observed among various types of drug dependences (*25*) and the known hyperglutamatergic state associated with alcohol withdrawal (*26*), we hypothesized that short-term alcohol abstinence would also be associated with lower cerebral mGlu5 availability. Additionally, we addressed whether a potential lower mGlu5 availability in alcohol-dependent patients is related to the amount of prior alcohol consumption and to craving.

MATERIALS AND METHODS

Participants

Patients with a DSM-IV diagnosis of alcohol dependence were recruited by a board-certified psychiatrist specialized in addiction at the Psychiatric Hospital Alexianen Tienen and the Psychiatry Department of the University Hospital Leuven (Belgium). All patients were included within the first 2 weeks of supervised abstinence. Exclusion criteria were any of the following: other psychiatric diseases (including first-degree relative), substance use disorders except nicotine dependence, chronic use of benzodiazepines, and abnormal findings on physical examination, blood test (with a tolerance for liver tests of ≤2 times the limit value), urine toxicology (Multi Urine Drugcontrol cassette, Ultimed Products, Germany) or structural magnetic resonance imaging. According to the local clinical procedure, 6 patients temporarily received clorazepate (mean cumulative dose, 100 mg; range, 50-225 mg) to relieve withdrawal symptoms.

Healthy controls were recruited through local advertisement and underwent a full medical and mental assessment at study intake. This sample represents a subsample of a previous study (*27*) and was randomly selected based on age by a person blind to both study protocols in order to obtain two age-matched controls for each patient (Table 1). Subjects reporting alcohol consumption >7 units/week (one unit=10 g alcohol) or regular binge drinking (≥5 units/occasion) were excluded. Temperament traits were assessed using the 240-item Cloninger Temperament and Character inventory (*28*).

The study protocol was approved by the Ethics Committee of the University Hospital Leuven. All participants signed a written informed consent

Assessment of Alcohol Use and Craving

Alcohol consumption in the prior 3 months was determined subjectively by the Time-Line Follow Back method (*29*). Excessive alcohol use was further assessed using the Alcohol Use Disorders Identification Test (AUDIT) (*30*). Moreover, quantitative analysis of alcohol consumption was performed via hair ethyl glucuronide (hEtG) analysis (*31*), providing a quantitative measure of alcohol consumption over the prior 3 months (*32*).

Alcohol craving in recently abstinent patients was assessed at the time of PET using the 13-item Dutch version of the Desire for Drugs Questionnaire (DDQ) adapted to alcohol (*33*).

mGlu5 PET Imaging and Radiometabolites Analysis

All subjects were asked to fast for 3 hours. Lack of alcohol consumption in the prior hours was verified by measuring breath alcohol concentration using a hand-held electronic device (Dräger Alcotest 6820, Luebeck, Germany). The radiometabolite analysis procedure has been described in detailed previously (*20*).

^{18}F-FPEB PET data were dynamically acquired on a HiRez Biograph PET/CT camera (Siemens Inc, Erlangen, Germany) for 90 min following bolus injection of ^{18}F-FPEB (Table 1). PET data were analyzed using PMOD (v3.605, PMOD Technologies, Zurich, Switzerland) and statistical parametric mapping (SPM v12). The ^{18}F-FPEB total distribution volume (V_T, as defined by Innis et al. (*34*)) was used as a surrogate for mGlu5 availability. To obtain V_T map, the Logan graphical approach (*35*) was used. For SPM analysis, V_T images were spatially normalized to the Montreal Neurological Institute space. Additionally, voxel-based findings were corroborated with a volumes-of-interest (VOIs) analysis using the N30R83 Hammers probabilistic atlas (*36*). VOI-based V_T values were derived from a reversible two-tissue compartment model (*20*,*37*). To control for the confounding effect of potential brain atrophy, voxel-based and VOI-based PET data were

corrected for partial volume effects using Muller-Gartner (*38*) and Geometric Transfer Matrix (*39*) methods, respectively.

Structural Magnetic Resonance Imaging and Brain Atrophy Assessment

A structural brain magnetic resonance imaging was acquired on a 3-Tesla scanner (Philips Ingenia, Healthcare, The Netherlands) for automatic VOI determination and co-registration with PET images. Additionally, potential brain atrophy in patients was assessed both at the voxel level (voxel-based morphometry) and at the VOI level by comparing groups' relative grey matter volumes.

Genotyping

Based on previous studies (*23,24*), the genotype for mGlu5 polymorphisms rs3462, rs3824927, rs308787, rs11020526, rs7931721 and rs495695 was determined in all participants (Supplemental Materials).

Statistical Analysis

Conventional statistical analyses were performed using Statistica v12 (Statsoft, Tusla, Oklahoma). Normally distributed variables are reported as mean±standard deviation and skewed variables as median (interquartile range). Independent samples t-test or Mann-Whitney U test were used, as appropriate. Based on the suggested involvement of mGlu5 in normal aging (*40*) (*27*), age was included as covariate in all analyses.

A SPM group comparison analysis was performed with smoking status as nuisance variable. V_T images corrected for partial volume effects were smoothed using a Gaussian kernel of 12 mm. The statistical threshold was set at $P<0.05$, corrected for family-wise error (P_{FWE}). To minimize the

chance of type I error in the secondary VOI-based analysis, only VOIs corresponding to regions with significant P_{FWE} were considered. VOI-based group comparison were analyzed using a multivariate general linear model with ^{18}F-FPEB V_T values as dependent variables and group status, smoking status and age as independent predictors. The effect of mGlu5 polymorphisms, withdrawal treatment and abstinence period were tested by adding genotyping data, the received benzodiazepine dose and days of abstinence into the model, respectively.

In patients, associations between VOI-based mGlu5 availability and self-reported alcohol use, hEtG levels and craving measurements were assessed using partial correlation coefficients with age as nuisance variable since both mGlu5 density (*27*) and alcohol craving (*41*) may be influenced by age.

RESULTS

Participant Characteristics

Participant characteristics are reported in Table 1. The two groups differed only for alcohol and nicotine use and the temperament trait harm avoidance.

Brain Atrophy in Patients

Compared to controls, voxel-based morphometry revealed lower gray matter volume in alcohol-dependent patients in the orbitofrontal cortex, straight gyrus, inferior/middle/superior temporal lobe, hippocampus, insula and putamen bilaterally (Fig. 1A). Consistently, evidence of brain atrophy in patients was observed in the additional VOI-based analysis (Fig. 1B).

Group Differences in mGlu5 Availability

All scans were performed at least 2 days from the last alcohol consumption (mean time interval, 7 d; range, 2-14 d) and at more than 24 h from the last clorazepate dosing (mean time interval, 91 h; range 25-186 h).

After correction for partial volume effects and controlling for age and smoking status, SPM showed a significant lower mGlu5 availability in alcohol-dependent patients in various, mostly limbic, cortical and subcortical brain areas (Fig. 2, Supplemental Table 1). These findings were confirmed by the VOI analysis, detecting a lower mGlu5 availability in alcohol-dependent subjects ranging from 14% (posterior cingulate cortex) up to 36 % (caudate nucleus) (Fig. 3). Noteworthy, no significant ^{18}F-FPEB V_T differences were observed between smokers and non-smokers in alcohol-dependent subjects in any region (global V_T=22±4 in smokers and 19±2 in non-smokers; P=0.8). Finally, similar highly significant ^{18}F-FPEB V_T differences were observed using data uncorrected

for partial volume effects, supporting that group differences in brain volumes did not affect the present PET findings. No significant sex differences in mGlu5 availability were observed, conform to previous studies (*27,42*). Regarding mGlu5 polymorphisms, we found no significant effects of an individual polymorphism genotype on ^{18}F-FPEB V_T in either the control or the patient group.

Correlation Between mGlu5 Availability and Alcohol Consumption

In alcohol-dependent patient, mGlu5 availability in the thalamus, insula, superior frontal gyrus, superior temporal gyrus and inferolateral temporal lobe was negatively correlated with hEtG concentration (Table 2, Fig. 4A). Concordant findings were observed at the voxel level using multiple regression analysis, except for the thalamus ($P<0.001$; Fig. 5). In contrast, no associations were found between mGlu5 and the self-reported alcohol consumption assessments. Additionally, no associations between mGlu5 availability and the duration of alcohol consumption or the time of abstinence were observed.

Correlation Between mGlu5 Availability and Alcohol Craving

Using age as nuisance variable, VOI-based mGlu5 availability in patients correlated positively with the craving item '*negative reinforcement*' in the cingulate cortex, middle frontal gyrus and inferolateral parietal lobe (Table 2, Fig. 4B-E). These results did not survive a Bonferroni correction for multiple testing and were not reproduced by SPM analysis, which may be due to the low effect size and the reduced sensitivity of the SPM compared to the VOI-based analysis, respectively.

DISCUSSION

To our knowledge, this is the first study investigating mGlu5 availability in alcohol-dependent patients *in vivo*. Within 2 weeks of supervised abstinence, alcohol-dependent patients demonstrated lower mGlu5 availability in mainly limbic areas. Moreover, mGlu5 availability was inversely correlated with the quantitative measure of recent alcohol consumption. Finally, we found a positive relationship between mGlu5 and alcohol craving during early abstinence, supporting the clinical significance of the present findings.

In patients, we observed reduced mGlu5 availability in corticolimbic regions in which long-lasting neuroadaptations are suggested to occur upon extensive alcohol exposure (*1*,*26*). A lower cerebral mGlu5 availability was also observed in nicotine- (*43*) and cocaine-dependent (*44*) patients, supporting the hypothesis that mGlu5 downregulation represents a common neuroadaptation occuring across various types of substance dependence. In alcohol dependence, a well-described neuroadaptation is the up-regulation of N-methyl-D-aspartate receptor expression and function, which contributes to the hyperglutamatergic state associated with short-term abstinence (*45*) and in turn, to neurotoxicity (*46*), anxiety (*47*), craving (*48*) and relapse (*49*). Therefore, given the functional positive coupling of mGlu5 and N-methyl-D-aspartate receptor (*3*), mGlu5 availability might serve as a compensatory mechanism to attenuate this hyperglutamatergic state by reducing postsynaptic excitability. On the other hand, given its critical role in adaptive learning (*50*), impaired mGlu5-dependent plasticity may contribute to the chronic inability of dependent individuals to extinguish drug-associated memories and to form adaptive behaviors to escape drug seeking and taking (*51*,*52*).

In patients, the finding of a negative association between hEtG concentrations and mGlu5 suggests a dose-response relationship to alcohol exposure. In contrast, no significant associations

were found with self-reported alcohol measures. This discrepancy might be due to the difficulty in recalling accurate amounts of consumed alcohol in presence of cognitive impairments and/or due to the well-known tendency to underestimate or inaccurately report one's own alcohol use (*53*).

An important consideration to address is whether the present findings have some clinical implications. A fundamental component of alcohol dependence is craving. Using a subjective assessment of craving (*54*), we found a positive relationship between the craving dimension '*negative reinforcement*' and mGlu5 availability in specific regions that are involved in controlling drug craving (*55,56*). This association between mGlu5 and craving is also in line with preclinical evidence showing that antagonism of mGlu5 reduces the escalation of alcohol use as well as anxiety-like (*57*) and relapse-like behaviors (*58*). Therefore, our findings support the potential of specific mGlu5 negative modulators to reduce craving and relapse in alcohol-dependent patients (*59*).

Some limitations of this study need to be mentioned. First, the sample size of alcohol-dependent patients was small, though large enough to draw robust conclusions regarding group difference in mGlu5 availability. In contrast, the correlations with craving showed small effect size and this finding should be seen as preliminary. Second, considering the hyperglutamatergic state associated with alcohol withdrawal (*26*), group differences in extracellular glutamate levels potentially confound findings. Indeed, although binding to an allosteric site, radioligand displacement by endogenous glutamate might occur by other mechanisms than direct competition (*60*). Yet, we previously found no association between ACC ^{18}F-FPEB V_T and glutamate levels, supporting no major effects of endogenous glutamate on ^{18}F-FPEB binding. Third, this study cannot disentangle whether the reduced mGlu5 availability in patients represents a pre-existent condition or a consequence of chronic alcohol use, which would be better investigated in longitudinal studies. Finally, mGlu5 scans have been performed within a rather large withdraw

time window (2-14 d). In view of the preclinical literature suggesting that mGlu5 expression varies depending on the time of abstinence and also the brain regions being studied (*6-9*), more studies are needed to investigate how mGlu5 expression regionally adapt over time in alcohol-dependent patients.

CONCLUSION

In conclusion, we found that alcohol-dependent patients have lower corticolimbic mGlu5 availability compared to controls. In specific regions, this reduction correlated negatively with the amount of recent alcohol consumed and positively with the negative reinforcement dimension of craving. These findings provide the first *in vivo* human evidence for a central role of mGlu5 in the neuroadaptations underlying alcohol dependence and support the potential of mGlu5 antagonists for the prevention or reduction of craving in patients. Future follow-up mGlu5 PET studies in the course of alcohol detoxification are warranted to assess both the potential reversibility of lower mGlu5 availability and the potential of mGlu5 PET for individual prediction of relapse.

DISCLOSURE

Authors report no biomedical financial interests or potential conflicts of interest.

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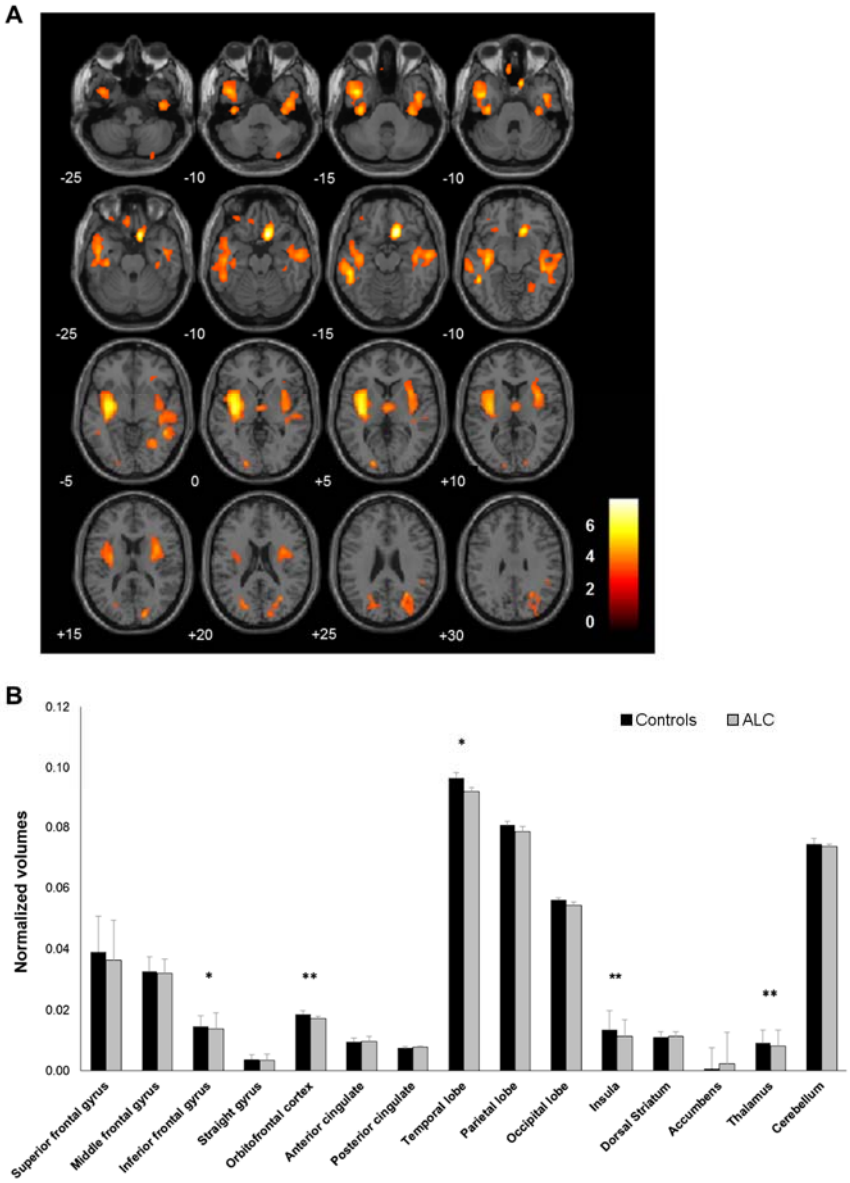

Figure 1. Lower gray matter volumes in alcohol-dependent patients compared to controls. (A) Statistical parametric mapping displayed at $P<0.001$ ($K_{EXT}>50$ voxels). (B) Volume-of-interest analysis.*$P<0.05$; **$P<0.001$.

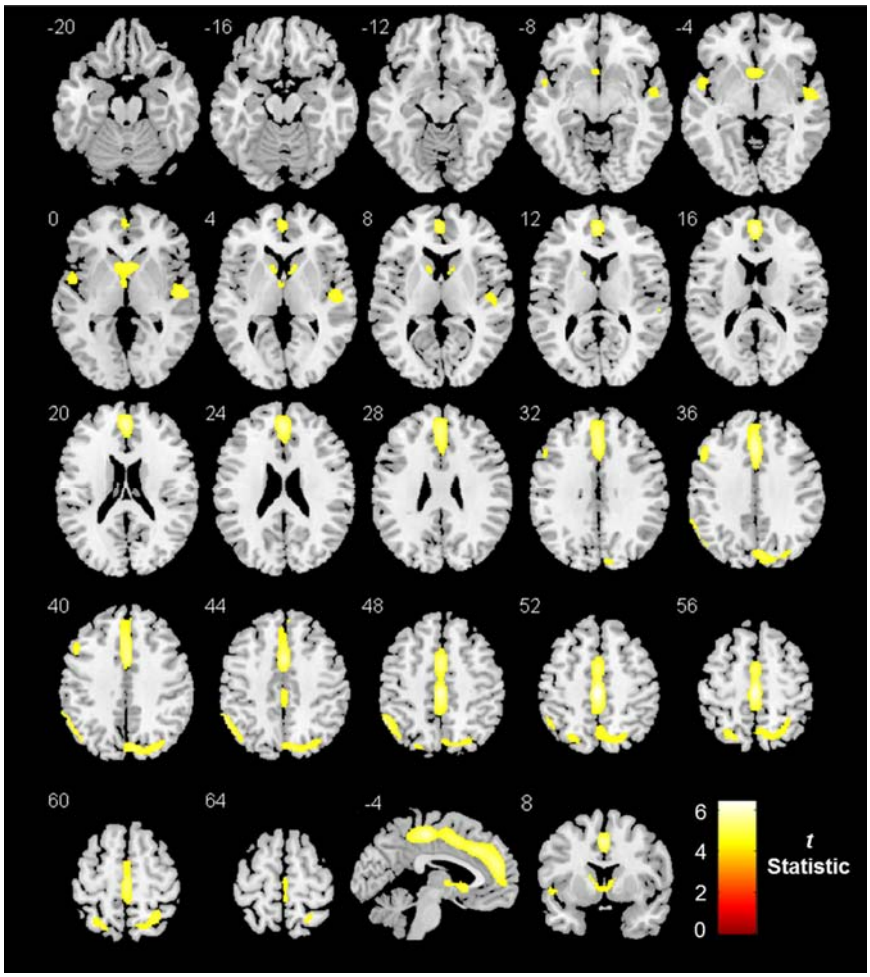

Figure 2. Lower mGlu5 availability (^{18}F-FPEB V_T) in alcohol-dependent subjects versus controls. Statistical parametric mapping displayed at $P_{FWE}<0.05$ ($K_{EXT}>200$ voxels).

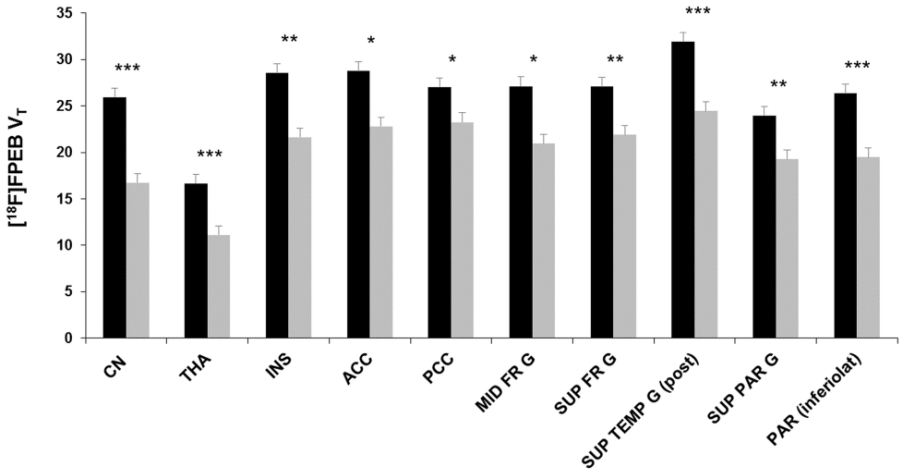

Figure 3. Volume-of-interest analysis of mGlu5 availability (^{18}F-FPEB V_T) in controls (black bars) versus alcohol-dependent subjects (gray bars). Bars and error bars are mean and standard deviation, respectively. CN, caudate nucleus; THA, thalamus; INS, insula; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; MID FR G, middle frontal gyrus; SUP FR G, superior frontal gyrus; SUP TEMP G (post), superior temporal gyrus, posterior part; SUP PAR G, superior parietal gyrus; PAR (inferolat), parietal lobe, inferolateral part. *P<0.05; **P<0.01; ***P<0.001.

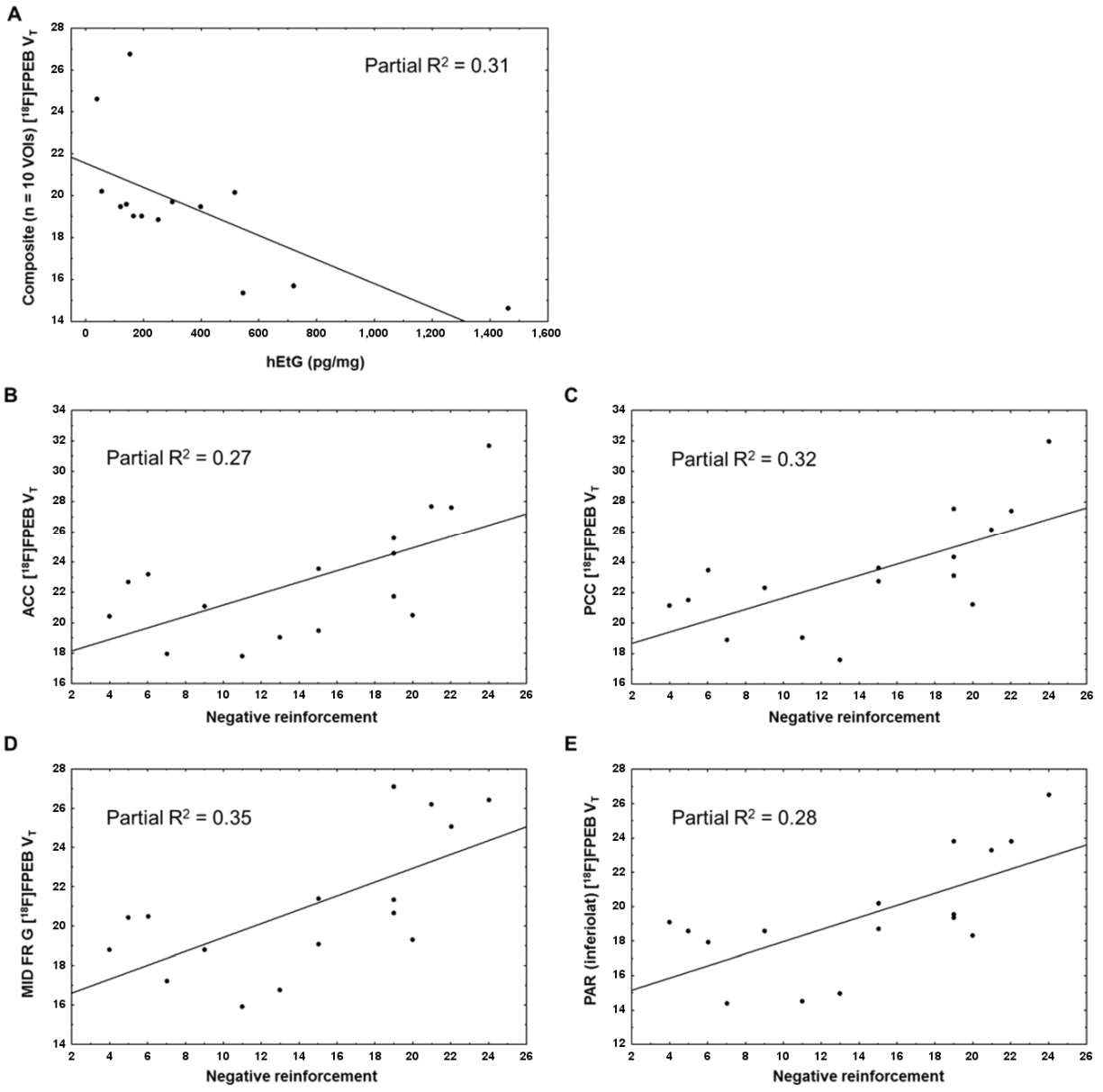

Figure 4. Alcohol-dependent subjects' scatterplots showing the relation (A) between mGlu5 availability (^{18}F-FPEB V_T) and hair ethyl glucuronide (hEtG) levels at a mean composite VOI, and (B, C, D and E) between mGlu5 availability and the *negative reinforcement* craving score for the anterior cingulate (ACC), posterior cingulate (PCC), middle frontal gyrus (MID FR G) and inferolateral temporal lobe.

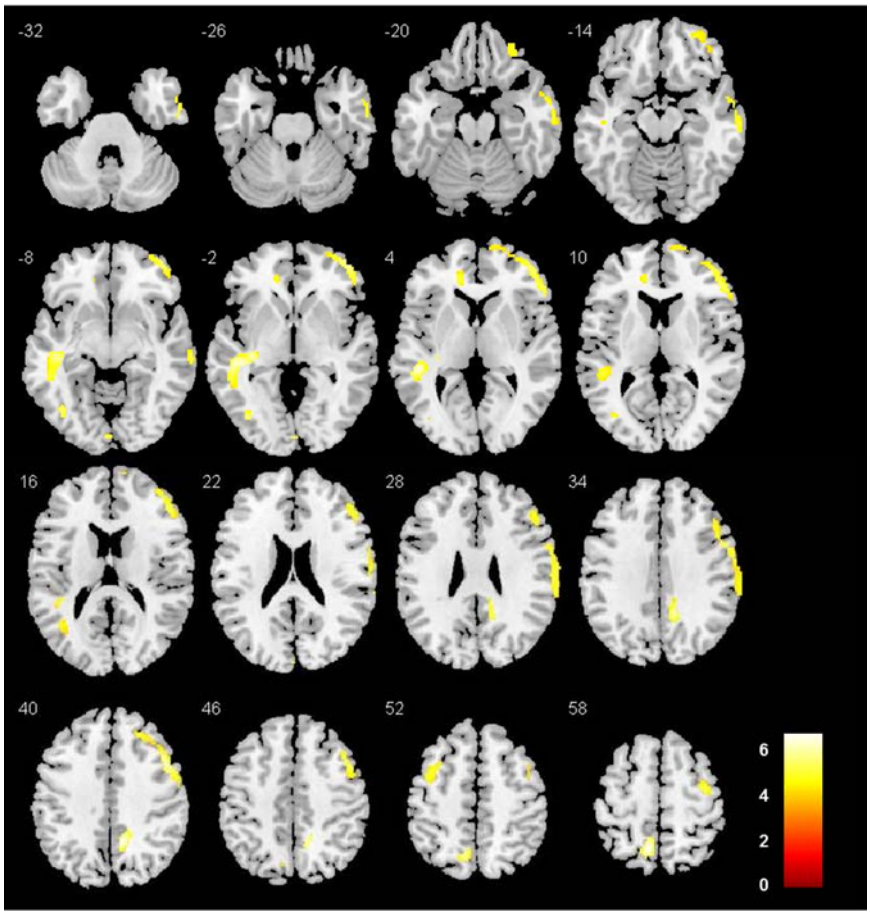

Figure 5. Statistical parametric mapping showing the negative association between mGlu5 availability (^{18}F-FPEB V_T) and hair ethyl glucuronide (hEtG) levels in alcohol-dependent patients (P<0.001, K_{EXT}>50 voxels).

Table 1. Overview of participant characteristics.

	Alcohol-dependent subjects (n=16)	**Controls (n=32)**	***P*-value**
Gender (female/male)	3/13	14/18	0.088
Age (years)	46±8	45±13	0.80
Body mass index (kg/m ^{2})	24.8±3.5	24.5±3.5	0.70
Education (years)	15.5±5.7	16.1±4.4	0.67
Temperament traits (TCI scores)			
Novelty-seeking	19.1±6.7	16.2±4.8	0.093
Harm avoidance	20.6±9.2	13.3±5.6	**0.001**
Reward dependence	15.8±4.7	17.5±4.2	0.22
Persistence	4.7±2.4	4.9±2.1	0.85
Family history of alcoholism (yes/no)	13/3	0/32	**<0.001**
Alcohol use			
Reported intake (grams/week)	1627±648	24±21	**<0.001**
Hair ethyl glucuronide level (pg/mg)	221.5 (375.1)	8.1 (12.2)	**<0.001**
AUDIT score	27±4	2±1	**<0.001**
Reported use (years)	26.5±12.9	26.6±8.1	0.99
Reported problematic use (years)	12.7±10.7	-	-
Abstinence (days)	7.3±4.0	-	-
Alcohol craving (DDQ scores)			
Desire and intention	15.7±7.0	-	-
Negative reinforcement	14.8±6.5	-	-
Control	4.0 (5.0)	-	-
Nicotine use			
Yes/no	11/5	0/32	**<0.001**
Number of cigarettes per day	18.5 (20)	-	-
Regular use (years)	23±6	-	-
FTND score	5.5±2.7	-	-
^{18}F-FPEB			
Injected activity (MBq)	175±11	177.0±7	>0.5
Specific activity (MBq/nmol)	97±51	90±52	>0.5
Injected mass (μg)	0.6±0.4	0.6±0.6	>0.5

AUDIT=Alcohol Use Disorder Identification Test; DDQ=Desire for Drug Questionnaire adapted for alcohol; FTND=Fagerstrom Test for Nicotine Dependence. Continuous data are mean±standard deviation or median (interquartile range) and dichotomous data are numbers.

Table 2. Partial correlation coefficients* of mGluR5 availability (^{18}F-FPEB V_T) versus recent alcohol consumption and craving in alcohol-dependent subjects.

Volume-of-interest	hEtG levels (pg/mg)		Alcohol craving (DDQ)					
			Desire and intention		Negative reinforcement		Control	
	r	***P***	***r***	***P***	***r***	***P***	***r***	***P***
Caudate Nucleus	-0.38	0.20	0.05	0.86	0.10	0.73	-0.15	0.58
Thalamus	**-0.65**	**0.03**	0.00	1.00	0.48	0.07	0.16	0.57
Insula	**-0.58**	**0.04**	-0.21	0.46	0.49	0.06	0.24	0.40
Anterior Cingulate Cortex	-0.43	0.04	-0.14	0.61	**0.52**	**0.05**	0.26	0.36
Posterior Cingulate Cortex	-0.45	0.12	0.04	0.88	**0.57**	**0.03**	0.18	0.51
MID FR G	-0.50	0.08	-0.10	0.73	**0.59**	**0.02**	0.18	0.52
SUP FR G	**-0.57**	**0.04**	-0.08	0.78	0.50	0.06	0.08	0.77
SUP TEMP G (post)	**-0.58**	**0.04**	-0.18	0.52	0.43	0.11	0.02	0.95
SUP PAR G	**-0.56**	**0.04**	-0.03	0.91	0.50	0.06	0.09	0.76
PAR (inferolat)	-0.52	0.06	-0.02	0.95	**0.53**	**0.04**	0.05	0.85
Mean (n=10 VOIs)	**-0.56**	**0.04**	-0.09	0.76	**0.53**	**0.04**	0.11	0.69

* with age as nuisance variable.

DDQ=Desire for Drug Questionnaire adapted for alcohol; hEtG=Hair Ethyl Glucuronide; MID FR G=middle frontal gyrus; SUP FR G=superior frontal gyrus; SUP TEMP G (post)=superior temporal gyrus, posterior part; SUP PAR G=superior parietal gyrus; PAR (inferolat)=parietal lobe, inferolateral part.

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